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Comparison of the Biological Effects in Rats of Radiation Sterilized and Autoclave Sterilized Food

by

W. H. Eriksen, W. Hjarde[x)], H. Lieck[x)], A. Juul and C. Emborg

Danish Atomic Energy Commission
Research Establishment Risø
Accelerator Department

Abstract

Three Groups of SPF Wistar rats were fed on radiation-sterilized, autoclave-sterilized and ordinarily autoclave-treated SPF food respectively. During 1 1/2 years' experimentation no significant differences between the groups were found with regards to general health, growth rate, feed consumption and reproduction performance. Mutagenic effects were not demonstrated either at autopsies or at a dominant lethal mutation test.

x) The National Food Institute, Copenhagen

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CONTENTS

1. INTRODUCTION

The wholesomeness testing project described in this report was initiated in the autumn of 1969. The investigations were discussed and parts were planned in collaboration with the following five institutes: 1) The National Institute of Public Health, Utrecht, The Netherlands, 2) The Institute for Atomic Sciences in Agriculture, Wageningen, The Netherlands, 3) The National Food Institute, Copenhagen, Denmark, 4) The Accelerator Department, Research Establishment Risø, Roskilde, Denmark, and 5) The Control Department, Statens Seruminstitut, Copenhagen, Denmark. The major objective was to develop more sensitive methods for quantitative assessment of the wholesomeness of irradiated commodities on a general basis.

Although wholesomeness research has been pursued for almost 20 years, general clearance of food irradiation has so far not been achieved. One fact may have been particularly important in delaying a general acceptance of the process: The health authorities have considered the irradiation process, with its production of radiolysis products, as being comparable to a process where an additive is admixtured to food. Accordingly, as the composition of various foods differs and consequently the radiolysis products also differ, the demand has been individual testing of each item of food under the specific circumstances. This demand has necessitated an almost insurmountable item-by-item testing.

The majority of investigations have compared the irradiated product with its non-irradiated counterpart only. This is not relevant. It seems more relevant to compare irradiated products with products preserved by conventional methods such as frying, cooking or smoking in order to establish the relative potential of all these methods to induce toxic compounds. As the aim was to detect possible toxic compounds, it was considered necessary to compensate for some of the nutritional losses occurring during the treatments in order to ensure nutritionally adequate food after the treatments.

These points were taken into consideration in the planning of the present experiments. Accordingly, a whole diet adapted to the recognized needs of SPF rats was chosen and radiation sterilized food was compared to two autoclaved foodstuffs (1) 110^{o}C at 10 min.; 2) 120^{o}C at 15 min.) of the same composition.

2. MATERIAL AND METHODS

The food used throughout the present series of experiments was a pelleted "Rostock Mixture" from Korn- og Foderstofkompagniet, Copenhagen. A given batch of food was used for a period of no more than 3 or 4 months. Before the experiments started, both the raw food and the food subjected to the three different sterilization procedures were analysed by the National Food Institute, Copenhagen, for the content of the following ingredients:

Total nitrogen; digestible nitrogen; threonine; cystine; methionine; valine; isoleucine; leucine; tyrosine; phenylalanine; lysine; histidine; arginine; tryptofane; glycine; alanine; serine; aspartic acid; glutamic acid; proline; fat; linoleic acid; vitamin A; vitamin B_1; vitamin B_2; vitamin B_6; vitamin B_{12}; niacin; free d-pantothenic acid; free folic acid; biotin; vitamin D; vitamin E; vitamin K_3; cholin chloride.

Based on the results of these analyses, the raw food was, prior to pelletization, enriched with appropriate amounts of compounds deficient in the sterilized diet (histidine, methionine, lysine, tryptofane, vitamin B_1 and vitamin A). The resulting foods met the requirements for laboratory rats recommended by the U.S. National Academy of Sciences, National Research Council (National Academy of Sciences 1962).

The experiments, which comprised 288 SPF Wistar rats, were carried out at the Møllegaard Breeding Laboratories in Ejby. These Wistar rats are derived from gnotobiotes from the Zentralinstitut für Versuchstierzucht, Hannover. The animals were divided into two main groups, A and B; the experiments started with an interval of 1 1/2 months. Two control groups (A_1 and B_1) were fed conventionally treated food for SPF animals (saturated steam for 10 min. at 110°C), two groups (A_{II} and B_{II}) received food autoclaved to meet hospital standards (saturated steam for 15 min. at 120°C), and two groups (A_{III} and B_{III}) received food irradiated with a dose corresponding to a 12D botulinum inactivation (5 Mrad). All animals were derived from parents that had been fed the respective diets two weeks prior to mating. Mating was undertaken through 72 hours, one male to four females. Five days after delivery, the litters were reduced to four males and four females. The following parameters were observed: litter size, growth rate, feed consumption and general health.

Three months after weaning, one third of the animals (randomly selected) were killed; autopsies were performed and the weights of a number of body organs were determined. These and other organs were preserved in formaldehyde solution for any later studies. Histological examinations were per-

formed on a few randomly selected animals and on all animals with abnormal findings at the autopsy.

The remaining live animals in every group were at random divided into two subgroups. The parameters observed in subgroup one (longevity group) are: longevity, general health, cause of death and pathological changes. The parameters observed in subgroup two (reproduction group) further comprise fertility, litter size, litter weight at birth and at weaning, number of stillborns and mortality among the pups. Matings were undertaken every two months. Males and females were kept together through seven days. The litters were not reduced and were followed until they were weaned, i. e. until they are 21 days old.

3. RESULTS AND DISCUSSION

3.1. Diet Analyses

Table 1 shows the composition of the rat feed "Rostock Mixture" as declared by the manufacturing company A/S Korn- og Foderstofkompagniet, Copenhagen.

Table 2 shows some results from the feed analyses undertaken at the National Food Institute, Copenhagen. The table shows that the amino acid content and the true digestibility of the proteins are almost unaffected by the radiation in the samples analysed, while the contents of certain vitamins, particularly vitamins A-B_1-B_6-B_{12}-E and folic acid have decreased (roughly 20 to 40%). In other samples similar decreases in the K_3-vitamin content and only very slight decreases in E-vitamin content were found. Consequently, the raw feed was enriched in order to make sure that the sterilized products fulfil the NAS-NRC requirements for rats. All batches of feed have been analysed similarly. The contents of vitamin A-B_{12} and E, which in the first batch were only present in marginal amounts, were determined as function of storage time (three months). No significant changes have been observed.

3.2. Growth Performance

The growth rates of the pups are shown in figs. 1 and 2. The performance was normal for this strain. For the A-groups, the growth rate for the males was slightly lower for the A_{II} group fed diet autoclaved at 120°C than in the other two groups. However, this difference is not considered significant since the bodyweight of the A_{II} group is within the range of vari-

ations of the bodyweights of the other two groups. Furthermore at weaning, when the animals were randomly chosen, the bodyweight of the A_{II} males were by chance some per cent lower than those of the other two groups. This difference expressed in percentages was maintained throughout their lifetime. It was even enlarged when the reduction of the numbers of animals at the age of 160 days incidentally caused a decrease in the average weight of the remaining males in the A_{II} group.

Food consumption in the various groups was at the same level, which may be seen from figs. 3 and 4.

3.3. Reproduction Performance

Extensive studies of the reproduction performance were undertaken. Groups were set up for continuous breeding. Every two months litters were produced in the three groups and various parameters were followed until weaning. Table 3 shows some of the results obtained in eleven mating cycles, six in the A-groups and five in the B-groups. Totally 244 matings have presumedly taken place resulting in 213 litters containing 2000 pups. All parameters followed lie within the range of the satisfactory standards of reproductive performance of the laboratory rat as laid down by the U.S. National Academy of Sciences - National Research Council (Nutrient Requirements of Laboratory Animals, 1962, No. 10). The animals for the reproduction groups were selected at random and consequently no fertility tests were undertaken before the initiation of the scheme. Non-fertile animals have been registered in group II (autoclave-sterilized food, 120°C), and group III (radiation-sterilized food, 5 Mrad). Out of 16 females and 16 males in each group we found in group II one non-fertile female and one non-fertile male. In group III two males were found to be non-fertile.

As may be seen from table 3 no significant differences between the groups were found.

In order to increase the sensitivity of the reproduction investigations, a test was carried out which is assumed to be specifically sensitive to detecting dominant lethal mutations. Pregnant females (pups derived from the three groups) were autopsied 15 to 17 days after the midweek of presumptive mating. Corpora lutea, deciduomata, dead and living embryos were counted. The induction of dominant lethal mutations was evaluated by comparing the ratio of living embryos with the number of corpora lutea in the three groups. No indication of dominant lethal mutations being induced in the group fed on radiation sterilized food has been found. (Int. J. Radiat. Biol., 1972, Vol 22, No. 2, 131-135).

3.4. General Health

The state of health was high and there seemed to be no difference between the groups in that respect. Table 3 shows that the weaning percentage of the live born pups is approximately 98%, which is far in excess of the NAS-NRC requirements for a satisfactory standard (90%). The weights of a number of body organs determined at the autopsies three months after weaning are presented in tables 4 and 5. No significant differences between the three groups were encountered. At an age of approximately 1 1/2 years the remaining animals were killed. The autopsies did not reveal any significant differences between the three groups. From weaning and throughout the whole experimental period only 7 animals out of 288 (2.4%) died or were killed because of diseases (table 6).

The total number of animals with diagnosticated autonomous processes amounts to five. One animal in group I (control food) had leucemia and two animals in each of the other two groups had each a well-defined, noninfiltrative tumor in the cutaneous or subcutaneous tissue (lipoma or fibroma).

4. CONCLUSION

Although 100% of the diets were treated with the sterilizing agents at rather high doses, no adverse effects were encountered. The experiments have revealed no significant differences between the three groups with regard to general health, growth rate, food consumption and reproduction performance. Mutagenic effects were not demonstrated either at autopsies or at a dominant lethal mutation test.

5. ADDENDUM

The investments in funds and manpower in the present project have been very modest in comparison with the wholesomeness testing programmes carried out elsewhere.

The investigations have had the practical implications of demonstrating that irradiated food for laboratory animals has certain practical advantages, and several laboratories in Denmark are now using this method. Such practical use effectively contributes to the accumulation of relevant wholesomeness data about radiation sterilized food.

Table 1

Rat Food "Rostock Mixture"

Manufacturing Company: "A/S Korn- og Foderstofkompagniet", Copenhagen

Guarantee of contents: 16% digestible Pure Protein
96 Feed Units per 100 kg
(calculated as for large farm animals)

Composition:

Wheat	11.50%
Oats, shelled	30.00%
Maize corn	30.00%
Fish meal	8.00%
Soyabean grits	9.00%
Dried yeast, min. 40% Crude Protein	3.00%
Lucerne grean meal	2.00%
Skim milk powder	3.00%
Vitamin-mineral mixture 1)	1.50%
Red mineral mixture 2)	2.00%
	100.00%

1) Vitamins mentioned below are mixed into:

84.68%	Wheat bran
2.50%	Ferro sulphate
0.50%	Cupri sulphate
6.90%	Zinc sulphate monohydrate
2.82%	Manganese carbonate
0.10%	Potassium iodide
2.50%	Ethoxyquin (Santoquin)

2) Red mineral mixture:

83.00%	Calcium hydrogen phosphate
15.00%	Sodium chloride
1.40%	Ferro sulphate
0.30%	Manganese oxide
0.20%	Cupri sulphate
0.06%	Cobalt sulphate
0.03%	Zinc oxide
0.01%	Potassium iodide

The mixture is guaranteed the following vitamin contents per gram until seven months after the production day:

40 i.u.	Vitamin A
10 i.u.	Vitamin D_3
18 mcg	Riboflavin
18 mcg	D-pantothenic acid
40 mcg	Vitamin K
6 mcg	Thiamine
50 mcg	Niacinamide
200 mcg	Vitamin E
1000 mcg	Choline chloride
0.025 mcg	Vitamin B_{12}

Table 2

The National Food Institute, Copenhagen

Nutritive value of rat diet (Rostock mixture)

genuine and after sterilization in three different ways

Nutrient	Units	Way of sterilization			
		Not sterilized	Irradiated	Autoclaved at 110° for 10 min.	Autoclaved at 120° for 15 min.
Total nitrogen	mg per gram	32.1	32.3	32.4	32.1
True digestability of nitrogen	%	78	77	72.5	71.5
Threonine	mg per gram	7.6	7.6	7.9	7.9
Cystine	" " "	3.20	3.20	3.10	3.05
Methionine	" " "	3.35	3.20	3.30	3.35
Valine	" " "	10.3	10.1	10.2	10.1
Isoleucine	" " "	8.7	8.6	8.4	8.6
Leucine	" " "	15.9	15.7	15.9	16.3
Tyrosine	" " "	5.9	5.6	6.1	6.2
Phenylalanine	" " "	9.1	9.0	9.0	9.4
Lysine	" " "	12.3	12.0	10.3	10.5
Histidine	" " "	5.1	4.9	4.7	5.0
Arginine	" " "	11.9	11.5	10.9	11.6
Tryptofan	" " "	2.3	2.3	2.3	2.3
Glycine	" " "	8.9	8.8	8.9	8.9
Alanine	" " "	10.5	10.3	10.4	10.5
Serine	" " "	8.9	8.8	8.9	8.8
Aspartic acid	" " "	16.6	16.8	16.7	16.7
Glutamic acid	" " "	33.0	33.3	35.2	34.5
Proline	" " "	10.5	10.9	11.9	11.3
Fat	%	4.8	5.0	4.8	4.9
Linoleic acid	%	1.80	1.85	1.90	2.05
Vitamin A	IU per gram	38	26	25	23
Vitamin B_1	µg per gram	9.7	7.8	5.8	3.85
Vitamin B_2	" " "	18	17	17	18
Vitamin B_6	" " "	5.3	3.5	3.8	3.5
Vitamin B_{12}	" " "	0.046	0.029	0.032	0.025
Niacin	" " "	190	170	190	190
d-pantothenic acid (free)	" " "	39	35	31	28
Folic acid (free)	" " "	3.5	2.7	3.4	3.0
Biotin	" " "	0.22	0.21	0.21	0.21
Vitamin D	IU per gram	16	19	8	12
Vitamin E	µg per gram	64	37	76	48
Vitamin K_3	" " "		less than 1		
Cholin chloride	mg per gram	3.50	3.35	3.35	3.25

Table 3

Results obtained in 11 mating cycles (A-groups 6 cycles, B-groups 5 cycles)

	Day 0			Day 21 (weaning)		
	I (110°C)	II (120°C)	III (5 Mrad)	I	II	III
Total number of presumed matings[x]	87	80	77			
Total number of litters	77	63	73			
Total number of pups	755	608	837			
Number of live pups	735	607	629	709	594	618
Number of stillborn pups per litter	0.26 ± 0.36	0.02 ± 0.04	0.11 ± 0.26			
Number of pups per litter	9.8 ± 0.8	9.7 ± 1.3	8.7 ± 1.8			
Number of pups per mating	8.7 ± 2.2	7.6 ± 1.8	8.3 ± 2.0			
Litter weight in grammes	56 ± 8	58 ± 7	51 ± 11	378 ± 28	360 ± 31	344 ± 42
Weight in grammes of female pups	5.7 ± 0.3	5.9 ± 0.3	5.8 ± 0.3	39 ± 3	38 ± 4	42 ± 7
Weight in grammes of male pups	6.0 ± 0.2	6.2 ± 0.3	6.2 ± 0.4	40 ± 3	40 ± 3	43 ± 6
Sex distribution in % Females Males	53 ± 6 47 ± 6	53 ± 7 47 ± 7	48 ± 6 52 ± 6			
Pregnancy %[x]	89 ± 20	79 ± 16	95 ± 10			
Mortality % day 0 - day 21				[xx] 2.1 ± 2.7	2.1 ± 2.0	1.7 ± 2.2

x) Two animals in group II and two animals in group III were found infertile. These cagings are not included in the table.

xx) One female was killed during this period. The eleven pups from this litter are not included in the table.

Table 4

Autopsies 3 months after weaning

Weights of Organs in g

	Males		
	Control FA_I	Autoclaved food FA_{II}	Irradiated food (5 Mrad) FA_{III}
2 Testicles	3.5 ± 0.2	3.4 ± 0.1	3.4 ± 0.2
2 Vesicula seminales + prostate	2.2 ± 0.2	1.9 ± 0.2	2.2 ± 0.3
Spleen	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
2 Kidneys	2.2 ± 0.2	2.4 ± 0.2	2.7 ± 0.3
Liver	12.3 ± 0.8	13.2 ± 1.6	12.7 ± 1.2
Thymus	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Heart	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
Brain	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1
Bodyweight	363 ± 33	342 ± 28	368 ± 20
	Females		
	Control FA_I	Autoclaved food FA_{II}	Irradiated food (5 Mrad) FA_{III}
Oviduct+ uterus	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Spleen	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
2 Kidneys	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
Liver	8.9 ± 0.1	8.8 ± 1.2	8.8 ± 2.7
Thymus	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
Heart	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
Brain	1.9 ± 0.1	1.9 ± 0.1	1.8 ± 0.1
Bodyweight	238 ± 12	212 ± 13	211 ± 14

Table 5

Autopsies 3 months after weaning

Weights of Organs in g

	Males		
	Control FB_I	Autoclaved food FB_{II}	Irradiated food (5 Mrad) FB_{III}
2 Testicles	3.34 ± 0.40	3.35 ± 0.15	3.39 ± 0.12
2 Vesicula seminales + Prostate	2.51 ± 0.23	2.23 ± 0.15	2.23 ± 0.32
Spleen	0.70 ± 0.10	0.64 ± 0.10	0.59 ± 0.02
2 Suprarenal glands	0.08 ± 0.01	0.09 ± 0.02	0.09 ± 0.04
2 Kidneys	2.63 ± 0.10	2.63 ± 0.21	2.92 ± 0.12
Liver	16.17 ± 1.66	15.59 ± 1.14	15.58 ± 1.03
Thymus	0.50 ± 0.21	0.41 ± 0.04	0.44 ± 0.03
Heart	1.20 ± 0.12	1.23 ± 0.12	1.20 ± 0.12
Brain	2.02 ± 0.10	2.02 ± 0.07	2.02 ± 0.04
Bodyweight	390.0 ± 25.4	371.5 ± 26.9	384.0 ± 12.1
	Females		
	Control FB_I	Autoclaved food FB_{II}	Irradiated food (5 Mrad) FB_{III}
2 Ovaries	0.19 ± 0.02	0.21 ± 0.10	0.16 ± 0.01
Uterus + Oviduct	0.64 ± 0.17	0.70 ± 0.17	0.66 ± 0.17
Spleen	0.54 ± 0.05	0.49 ± 0.10	0.46 ± 0.07
2 Suprarenal glands	0.09 ± 0.01	0.11 ± 0.03	0.10 ± 0.03
2 Kidneys	1.78 ± 0.17	1.63 ± 0.03	1.78 ± 0.14
Liver	9.20 ± 1.34	8.84 ± 0.55	8.05 ± 0.90
Thymus	0.38 ± 0.10	0.37 ± 0.03	0.38 ± 0.02
Heart	0.88 ± 0.08	0.80 ± 0.08	0.84 ± 0.08
Brain	1.88 ± 0.06	1.88 ± 0.06	1.83 ± 0.07
Bodyweight	230.0 ± 15.1	216.5 ± 7.5	224.8 ± 7.6

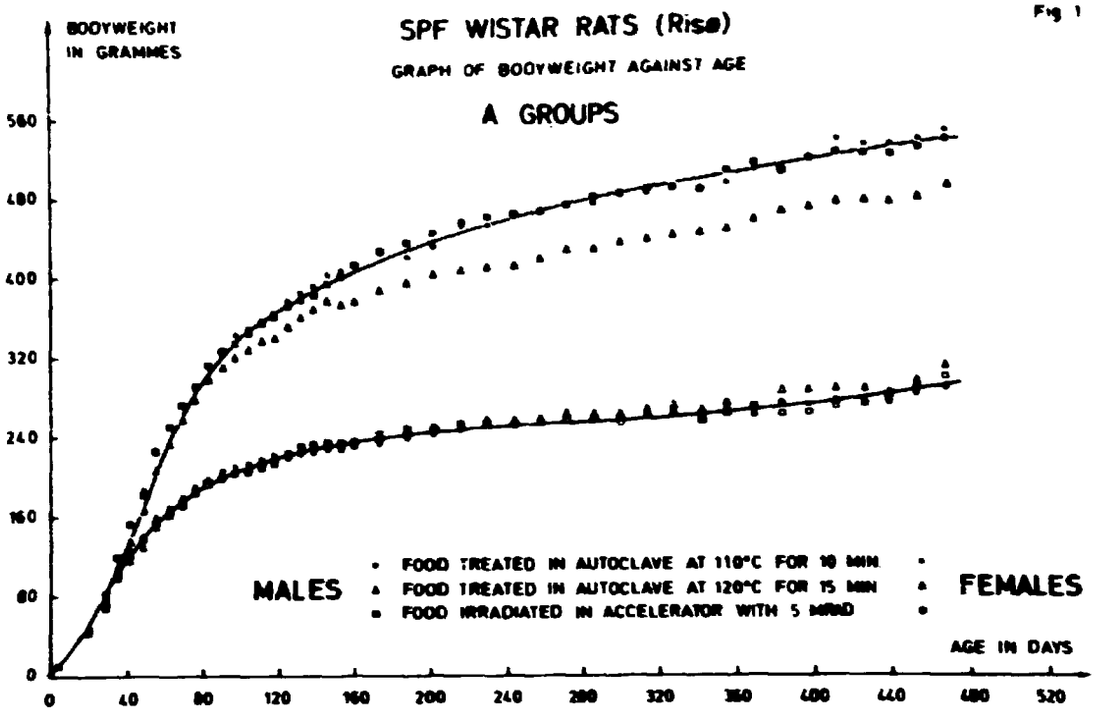
Table 6

Animals with fatal sicknesses during the experimental period (numbers in brackets)

Group	Food treatment	Total number of animals	Infections	Others
I	110°C	96	Peritonitis (1)	Leucemia (1)
II	120°C	96	Hepatitis (1)	
III	5 Mrad	96	Nephritis (1) Pneumonia (1) x) Wound (1)	Abnormous position of front teeth (1)

x) Had possibly the same ethiology as hairless areas commonly occurring in the animals of all groups.

Fig. 1


BODYWEIGHT
IN GRAMMES
SPF WISTAR RATS (Risø)
GRAPH OF BODYWEIGHT AGAINST AGE
A GROUPS
560
480
400
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240
160
80
0
MALES
FOOD TREATED IN AUTOCLAVE AT 110°C FOR 10 MIN
FOOD TREATED IN AUTOCLAVE AT 120°C FOR 15 MIN
FOOD IRRADIATED IN ACCELERATOR WITH 5 MRAD
FEMALES
AGE IN DAYS
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360
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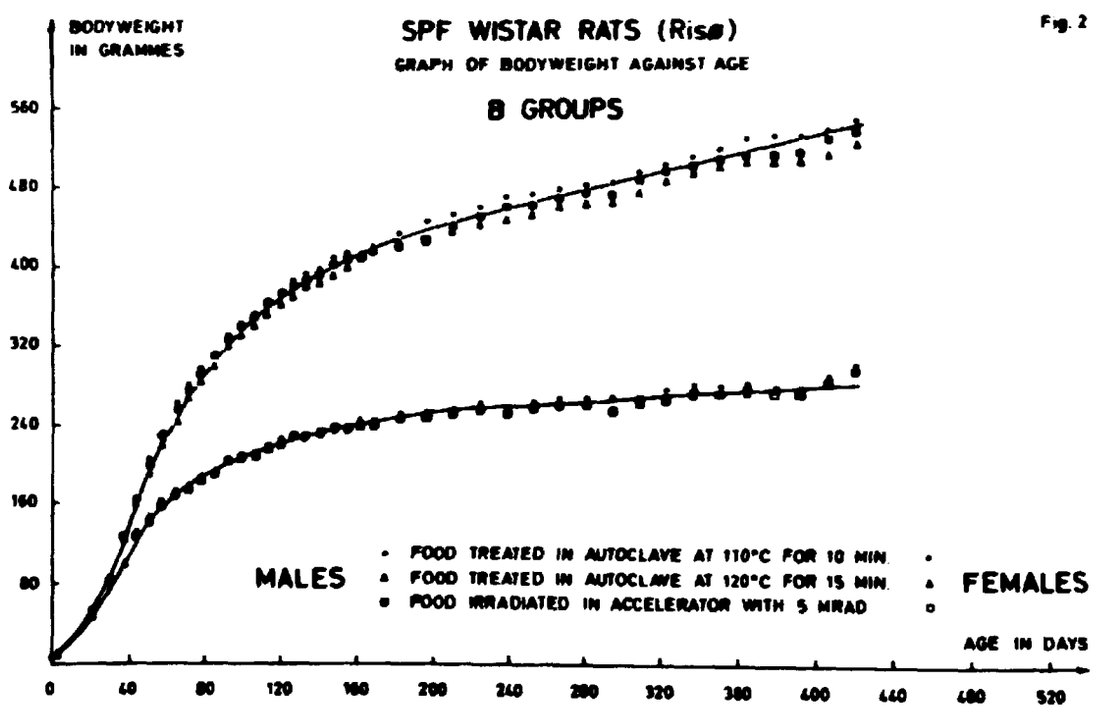
440
480
520

Fig. 2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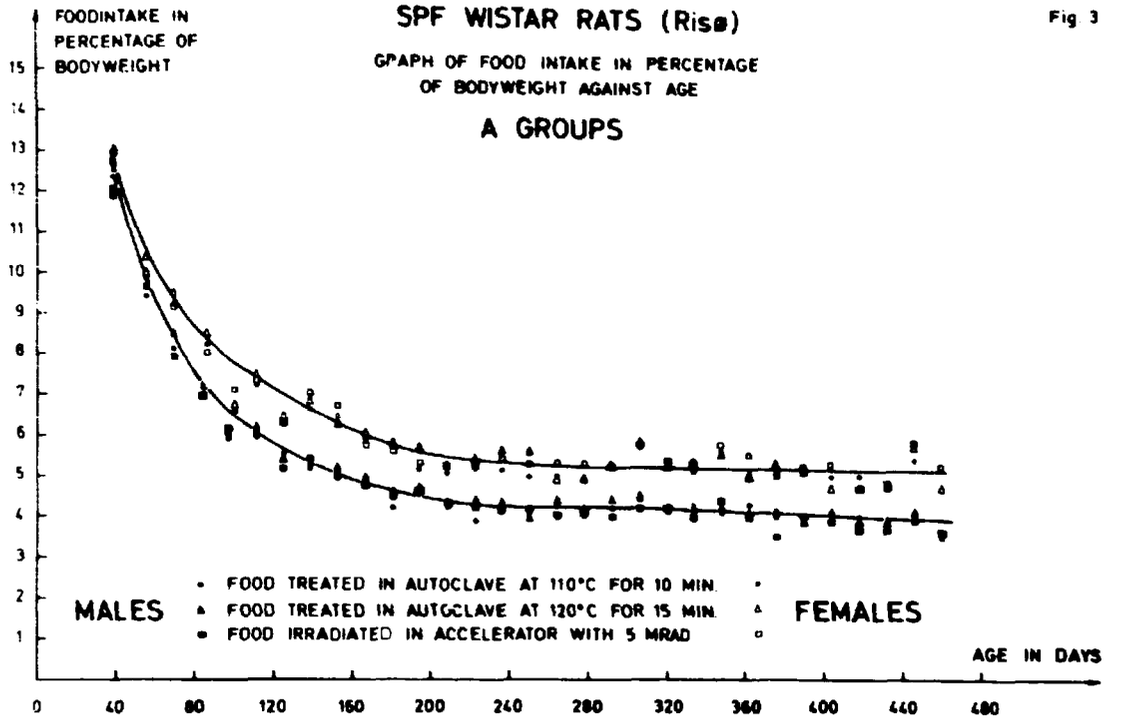
FOODINTAKE IN PERCENTAGE OF BODYWEIGHT
SPF WISTAR RATS (Risø)
GRAPH OF FOOD INTAKE IN PERCENTAGE OF BODYWEIGHT AGAINST AGE
A GROUPS
MALES
FEMALES
• FOOD TREATED IN AUTOCLAVE AT 110°C FOR 10 MIN.
▲ FOOD TREATED IN AUTOCLAVE AT 120°C FOR 15 MIN.
■ FOOD IRRADIATED IN ACCELERATOR WITH 5 MRAD
AGE IN DAYS
0 40 80 120 160 200 240 280 320 360 400 440 480

Fig 3

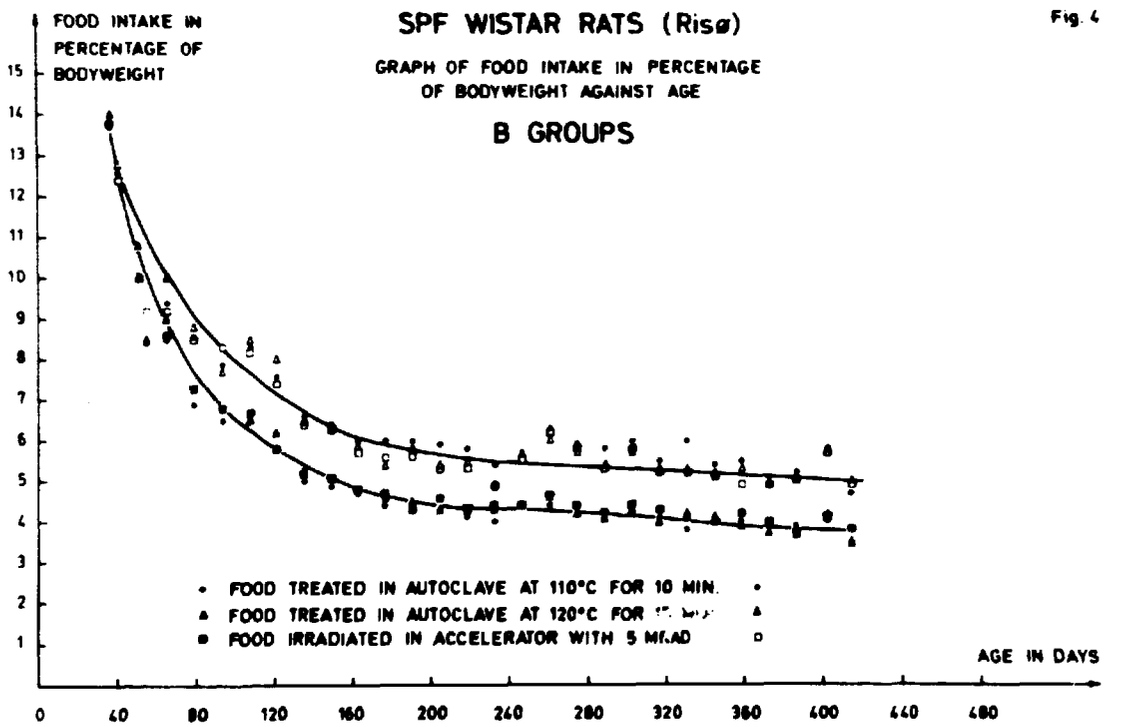
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Fig. 4

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